13,14-dihydro-15-keto Prostaglandin $F_{2\alpha}$ EIA Kit

Catalog No. 516671

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CONTENTS OF THE KIT

Number	Item	96 wells Quantity/Size	480 wells Quantity/Size
1	13,14-dihydro-15-keto $PGF_{2\alpha}$ EIA Antiserum	1 vial/100 dtn	1 vial/500 dtn
2	13,14-dihydro-15-keto $PGF_{2\alpha}$ AChE Tracer	1 vial/100 dtn	1 vial/500 dtn
3	13,14-dihydro-15-keto $PGF_{2\alpha}$ EIA Standard	1 vial/1 each	1 vial/1 each
4	EIA Buffer Concentrate	2 vials/10 ml	4 vials/10 ml
5	Wash Buffer Concentrate	1 vial/5 ml	1 vial/12.5 ml
5a	Tween 20	1 vial/3 ml	1 vial/3 ml
6	Mouse Anti-rabbit IgG Coated Plate	1 plate/1 each	5 plates/1 each
7	Plate Cover	1 cover/1 each	5 covers/1 each
8	Ellman's Reagent	3 vials/100 dtn	6 vials/250 dtn
14	EIA Tracer Dye	1 vial/1 each	1 vial/1 each
15	EIA Antiserum Dye	1 vial/1 each	1 vial/1 each

If any of the items listed above are damaged or missing, please contact our Customer Service department at (800) 364-9897 or (734) 971-3335. We cannot accept any returns without prior authorization.

PRECAUTIONS

WARNING: THIS PRODUCT IS NOT INTENDED OR APPROVED FOR USE IN HUMANS OR VETERINARY ANIMALS. RELIANCE ON THIS PRODUCT FOR ANALYTE MEASUREMENTS IN A THERAPEUTIC SETTING IS HAZARDOUS AND MAY RESULT IN ILLNESS OR INJURY.

- Please read these instructions carefully before beginning this assay.
- The reagents in this kit have been tested and formulated to work exclusively with ACE™ EIA kits. This kit may not perform as described if any reagent or procedure is replaced or modified.
- For research use only. Not for human or diagnostic use.

WARRANTY AND LIMITATION OF REMEDY

Cayman Chemical Company makes no warranty of any kind, expressed or implied, including, but not limited to, the warranties of fitness for a particular purpose and merchantability, which extends beyond the description of the chemicals on the face hereof, except that the material will meet our specifications at the time of delivery. Buyer's exclusive remedy and Cayman Chemical Company's sole liability hereunder shall be limited to refund of the purchase price of, or at Cayman Chemical Company's option, the replacement of, all material that does not meet our specifications. Cayman Chemical Company shall not be liable otherwise or for incidental or consequential damages, including, but not limited to, the costs of handling. Said refund or replacement is conditioned on Buyer giving written notice to Cayman Chemical Company within thirty (30) days after arrival of the material at its destination. Failure of Buyer to give said notice within said thirty (30) days shall constitute a waiver by Buyer of all claims hereunder with respect to said material.

IF YOU HAVE PROBLEMS

Our technical service staff may be reached by phone (800-364-9897, 734-971-3335), fax (734-971-3640), or E-Mail (techserv@caymanchem.com) Monday through Friday 8:00 AM to 6:00 PM EST. In order for our staff to assist you quickly and efficiently, please be ready to supply the lot number of the kit (found on the outside of the box).

Cayman Chemical offers an introductory course in EIA theory and practice. Please contact our Customer Service Department for more information.

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STORAGE AND STABILITY

This kit will perform as specified if stored at -20°C and used before the expiration date indicated on the outside of the box.

ADDITIONAL ITEMS REQUIRED

1. A plate reader with a 405-420 nm filter.
2. An adjustable pipettor.
3. A source of "UltraPure" water. Water used to prepare all EIA reagents and buffers must be deionized and free of trace organic contaminants ("UltraPure"). Use activated carbon filter cartridges or other organic scavengers. Glass distilled water (even if double distilled), HPLC-grade water, and sterile water (for injections) are not adequate for EIA. *[NOTE: UltraPure water is available for purchase (Catalog No. 400000).]*
4. Materials used for sample preparation (see page 6).

ABOUT THIS ASSAY

Prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) is one of the five primary prostaglandins derived enzymatically directly from the endoperoxide PGH_2. $PGF_{2\alpha}$ was initially discovered in seminal fluid, and to date the majority of the functional roles ascribed to it relate to fertility, pregnancy, and parturition. $PGF_{2\alpha}$ is a potent luteolytic agent and is used to induce ovulation in domestic livestock. It is also a potent uterine stimulant, and is part of the cascade of myometrial stimulants which induce and sustain labor. $PGF_{2\alpha}$ is rapidly metabolized to 13,14-dihydro-15-keto $PGF_{2\alpha}$ *in vivo*, by the enzymes 15-hydroxy prostaglandin dehydrogenase and Δ^{13}-reductucase.[1-4] Measurement of 13,14-dihydro-15-keto $PGF_{2\alpha}$ in plasma can be used as a marker of the *in vivo* production of $PGF_{2\alpha}$.[5,6]

INTRODUCTION TO ACE™ EIAs

Description of the ACE™ Competitive Enzyme Immunoassay[7]

This assay is based on the competition between 13,14-dihydro-15-keto $PGF_{2\alpha}$ and a 13,14-dihydro-15-keto $PGF_{2\alpha}$-acetylcholinesterase (AChE) conjugate (17-phenyl trinor $PGF_{2\alpha}$ tracer) for a limited number of 13,14-dihydro-15-keto $PGF_{2\alpha}$-specific rabbit antiserum binding sites. Because the concentration of the 13,14-dihydro-15-keto $PGF_{2\alpha}$ tracer is held constant while the concentration of 13,14-dihydro-15-keto $PGF_{2\alpha}$ varies, the amount of 13,14-dihydro-15-keto $PGF_{2\alpha}$ tracer that is able to bind to the rabbit antiserum will be inversely proportional to the concentration of 13,14-dihydro-15-keto $PGF_{2\alpha}$ in the well. This rabbit antiserum-13,14-dihydro-15-keto $PGF_{2\alpha}$ (either free or tracer) complex binds to the mouse monoclonal anti-rabbit IgG that has been previously attached to the well. The plate is washed to remove any unbound reagents, and then Ellman's Reagent (which contains the substrate to AChE) is added to the well. The product of this enzymatic reaction has a distinct yellow color and absorbs strongly at 412 nm. The intensity of this color, determined spectrophotometrically, is proportional to the amount of 13,14-dihydro-15-keto $PGF_{2\alpha}$ tracer bound to the well, which is inversely proportional to the amount of free 13,14-dihydro-15-keto $PGF_{2\alpha}$ present in the well during the incubation; or

$$\text{Absorbance} \propto [\text{Bound 13,14-dihydro-15-keto } PGF_{2\alpha} \text{ Tracer}] \propto 1/[\text{13,14-dihydro-15-keto } PGF_{2\alpha}]$$

A schematic of this process is shown in Figure 2 (see page 4).

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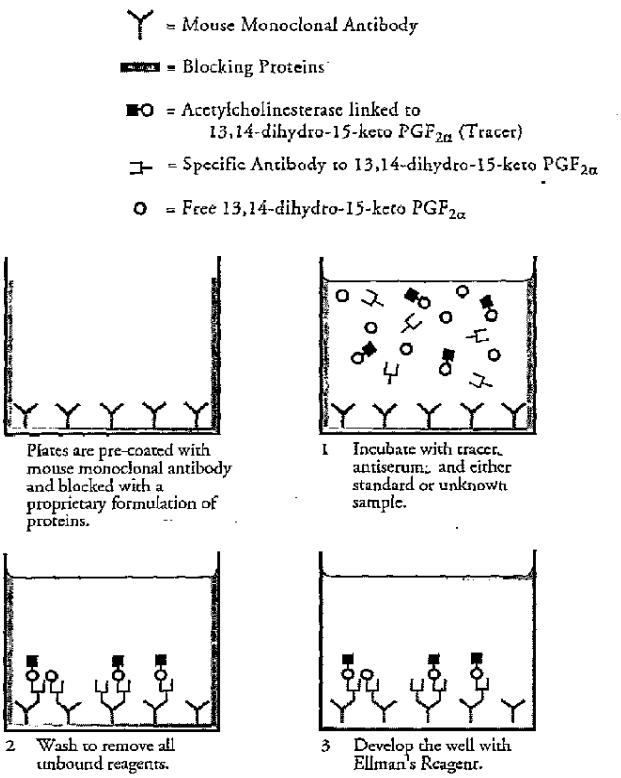

Figure 2. Schematic of the ACE™ EIA

Biochemistry of ACE™ EIAs

The electric organ of the electric eel, *Electrophorus electricus*, contains an avid AChE capable of massive catalytic turnover during the generation of its electrochemical discharges. The electric eel AChE has a clover leaf-shaped tertiary structure consisting of a triad of tetramers attached to a collagen-like structural fibril. This stable enzyme is capable of high turnover (64,000 s^{-1}) for the hydrolysis of acetylthiocholine.

A molecule of the analyte covalently attached to a molecule of AChE serves as the tracer in ACE™ enzyme immunoassays. Quantification of the tracer is achieved by measuring its AChE activity with Ellman's Reagent. This reagent consists of acetylthiocholine and 5,5'-dithio-*bis*-(2-nitrobenzoic acid). Hydrolysis of acetylthiocholine by AChE produces thiocholine (see Figure 3, page 5). The non-enzymatic reaction of thiocholine with 5,5'-dithio-*bis*-(2-nitrobenzoic acid) produces 5-thio-2-nitrobenzoic acid, which has a strong absorbance at 412 nm (ε = 13,600).

AChE has several advantages over other enzymes commonly used for enzyme immunoassays. Unlike horseradish peroxidase, AChE does not self-inactivate during turnover. This property of AChE also allows multiple development of the assay if it is accidentally splashed or spilled. In addition, the enzyme is highly stable under the assay conditions, has a wide pH range (pH 5-10), and is not inhibited by common buffer salts and preservatives. Since AChE is stable during the development step, it is unnecessary to use a "stop" reagent, and the plate may be read whenever it is convenient.

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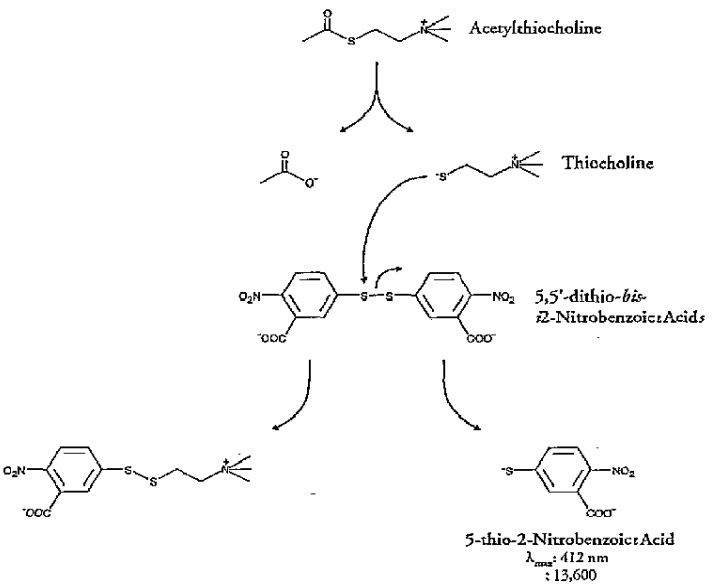

Figure 3. Reaction catalyzed by Acetylcholinesterase

Definition of Key Terms

Blank: background absorbance caused by Ellman's Reagent. Even freshly prepared Ellman's Reagent has some measurable absorbance, approximately 0.1 Absorbance Units (A.U.). The blank absorbance should be subtracted from the absorbance readings of all the other wells.

Total Activity: total enzymatic activity of the AChE-linked tracer. This is analogous to the specific activity of a radioactive tracer.

NSB (Non-Specific Binding): non-immunological binding of the tracer to the well. Even in the absence of specific antiserum a very small amount of tracer still binds to the well; the NSB is a measure of this low binding.

B_0 (Maximum Binding): maximum amount of the tracer that the antiserum can bind in the absence of free analyte.

%B/B_0 (%Bound/Maximum Bound): ratio of the absorbance of a particular sample or standard well to that of the maximum binding (B_0) well.

Standard Curve: a plot of the %B/B_0 values *versus* concentration of a series of wells containing various known amounts of analyte.

PRE-ASSAY PREPARATION

Water used to prepare all EIA reagents and buffers must be deionized and free of trace organic contaminants ("UltraPure"). Use activated carbon filter cartridges or other organic scavengers. Glass distilled water (even if double distilled), HPLC-grade water, and sterile water (for injections) are not adequate for EIA. UltraPure water may be purchased from Cayman Chemical (Catalog No. 400000).

Buffer Preparation *(Store all buffers at 4°C)*

1. EIA Buffer Preparation

   Dilute the contents of one vial of EIA Buffer Concentrate (vial #4) with 90 ml of UltraPure water. Be certain to rinse the vial to remove any salts that may have precipitated. *[NOTE: It is normal for the concentrated buffer to contain crystalline salts after thawing. These will completely dissolve upon dilution with water.]*

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